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ARTICLE

Evaluation of the Effect of Silylation in the Development of an Analytical Method for the Determination of UV Filters and Hormones by GC-MS

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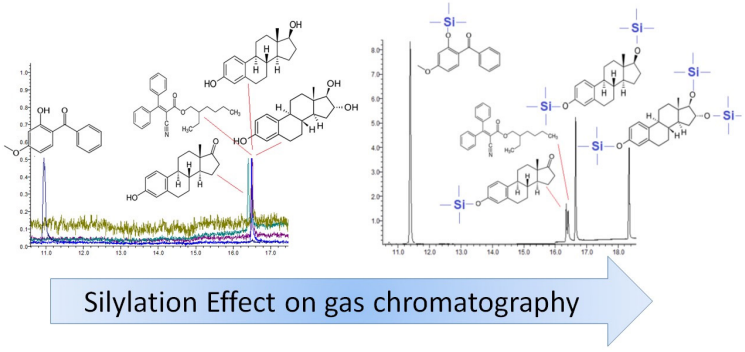

The objective of this work was to optimize a chromatographic method, combining derivatization (silylation) with MSTFA (*N*-methyl-*N*-(trimethylsilyl)trifluoracetamide) and analysis by gas chromatography coupled to mass spectrometry (GC-MS), for the determination of hormones and UV filters selectively, evaluating the effect of derivatization on the chromatographic response. The method developed for the qualitative analysis (SCAN mode) allowed the identification of the analytes more accurately, with similarities of the spectra superior to 80%. The limits of detection and quantification ranged from 0.1 to 1.3 μg L^{-1} and 0.3 to 4.2 μg L^{-1} respectively. The quantitative method, combined silylation with chromatographic determination in SIM mode, proved to be precise (Relative standard deviation <7.2%) and exact (relative error <2.0%), with models without lack of fit, and with correlation coefficients linear values greater than 0.9, in accordance with the requirements and standards of the regulatory bodies.

Keywords: derivatization, emerging contaminants, analytical validation, environmental chemistry, mass spectrometry

INTRODUCTION

The expression 'emerging contaminants' (ECs) refers to compounds detected in the soil, water, and air, both of anthropic origin (present in domestic, industrial and hospital effluents and those from agricultural and livestock activities) and of natural occurrence (present in different plant species, for example).[1] Among them are endogenous hormones, synthetic hormones, contraceptives, drugs of different compositions,

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caffeine, sucralose, nanomaterials, bactericides, insecticides, algaecides, herbicides, cleaning and personal hygiene products, sunscreens, water chlorination and ozonation products, among others, totaling more than a thousand compounds.[1,2] These products are not removed or eliminated by traditional water treatment processes for human consumption.[1,2]

The impacts of these ECs on the health of living beings are still not well understood, however several studies have been carried out, because these compounds present persistence and bioaccumulation in the environment. Some ECs, such as Pharmaceuticals and Personal Care Products (PPCP's), can enter the environment, mainly through wastewater and irrigation, being responsible for morphological, metabolic and even sexual alterations in aquatic fauna.[2]

Recently, substances used as organic ultraviolet filters (UV filters)[3,4] and estrogenic hormones[5-7] have drawn attention as emerging contaminants of interest due to their wide diffusion in the environment and potential adverse effects on the aquatic system and human life. Substances used as UV filters, such as octocrylene and oxybenzone, are used in PPCP's, food packaging and textile products to prevent photodegradation of polymers and pigments. Most organic filters are hydrophobic and degrade very less in water treatment plants.[8-11]

The relevance of estrogenic hormones in endocrine disruption is due to the high affinity of estrogens to receptors present in organisms of other species. It allows the action, even in concentrations of the order of ng L^{-1}, as the widespread excretion of these hormones occurs by humans and other animals in feces and urine; and the subsequent discharge of sewage, whether treated or not, into aquatic ecosystem.[12,13]

The concentration levels of octocrylene, benzophenone, estriol, β-estradiol, and estrone in environmental samples can vary depending on the location and specific conditions of the study. These compounds are generally found in very low concentrations in surface water, sewage treatment plant effluents, and sediments.[14]

Sun *et al*., using solid-phase extraction (SPE) with HLB cartridges and LC/MS-MS analysis, determined the distribution and abundance of PPCP's, considering seasonal variations in estuarine waters. It was found that benzophenone and octocrylene were present in more than 50% of the samples, with concentrations up to 532 ng L^{-1} during rainy periods for benzophenone and 31.6 ng L^{-1} for octocrylene during spring.[15]

The most used analytical techniques for quantification of ECs in environmental samples are based on methods that combine pre-concentration steps by Solid Phase Extraction (SPE) and chromatographic determinations.[16] Among them, gas chromatography coupled to mass spectrometry (GC-MS), gas chromatography with serial mass spectrometer (GC-MS/MS),[8] liquid chromatography with diode array detector (LC-DAD)[17] and liquid chromatography with mass spectrometry (LC-MS).[18,19] These techniques allow for the separation, identification, and precise quantification of the target compounds.

He *et al.*, combining SPE with LC-ESI-MS/MS, determined the levels of estrone (<0.2 μg L^{-1} to 2.0 μg L^{-1}), benzophenone-3 (31.1 μg L^{-1} to 113.7 μg L^{-1}), and octocrylene (11.9 μg L^{-1} to 43.77 μg L^{-1}) in the Chesapeake Bay waters (Maryland, USA).[8] In another study He *et al.*, developed a chromatographic method for the simultaneous determination of UV filters and estrogens by LC-MS/MS, establishing detection and quantification limits of 0.013 μg L^{-1} for estradiol and 0.005 μg L^{-1} for estrone, benzophenone-3, and octocrylene. This method was used for determinations in aquatic invertebrates.[14]

Sun *et al*., also reported the detection of benzophenone in effluent samples, with detection limits ranging from not determined to 8.70 μg L^{-1} for sample extracts obtained by SPE and analyzed by liquid chromatography with triple quadrupole detector.[15]

Chaves *et al.*, described the high frequency and levels of benzophenone-3 (>3–17 ng g^{-1}) among other drugs in sediment samples from the Anil and Bacanga rivers in northeastern Brazil. The analysis was performed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), using SPE and QuEChERS for water and sediment samples, respectively.[20]

Gas chromatography has been one of the most used techniques for the analysis of a wide range of compounds, as it uses both columns with polar and non-polar phases and is usually associated with mass spectrometric detectors (GC-MS), enabling the separation and identification of components in complex

mixtures.[21] Traditionally, determinations of polar compounds by GC-MS require post-extraction derivatization to improve thermal stability and reduce the polarity of the compounds. MSTFA has been widely used in the estrogenic steroid derivatization process due to its ease of use and low cost, which leads to the formation of trimethylsilyl derivatives (TMS).[22] Many studies have developed a method for specific classes of ECs such as drugs,[2,18] hormones[19,22,24] for the determination of hormones and sunscreens simultaneously by GC-MS. However, most studies use LC-MS.[8,13,25]

In their study, Sghaier *et al.* presented results for the analysis of the presence of hormones and other chemicals considered endocrine disruptors in water samples from six rivers. The samples, after being filtered and extracted using solid-phase extraction (SPE), were derivatized with N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) in the presence of the catalyst trimethylchlorosilane (TMCS), and the extracts were analyzed by GC-MS. Hormones found included 17 β-estradiol, estriol, and estrone, all with detection limits of 10 ng L^{-1}.[26]

Thurman *et al.,* present an analysis method for 17-β-estradiol, estrone, estriol, and other hormones. The method combines solid-phase extraction pre-concentration and on-column derivatization after injection into GC-MS.[27]

Moon *et al.,* developed a method that demonstrated linearity, precision, and accuracy for analysis using GC-MS with a high-temperature chromatographic column, which improved the detectability of 19 estrogens within 8 minutes. Among the analytes, estriol, β-estradiol, and estrone were identified and quantified. In the method, after solid-phase extraction, subsequent derivatization with pentafluoropropionyl (PFP) was performed.[28]

In this sense, the objective of this work was to develop an analytical method for the simultaneous determination of sunscreens, octocrylene (OC) and oxybenzone (BP-3) and the hormones estrone (E1), β-estradiol (E2) and estriol (E3), using derivatization for analysis by gas chromatography coupled to mass spectrometry.

MATERIALS AND METHODS

Instruments

The study was carried out with the aid of a gas chromatograph coupled to a mass spectrometer (Model QP2010 ULTRA – Shimadzu) equipped with an Rtx-5MS column (30.0 m x 0.25 mm d.i. x 0.25 µm – Ohio Valley Specialty Company, Marietta, Ohio, USA). The injection (1 µL) was performed in splitless mode with 2.0 mL min^{-1} purge and carrier gas flow (ultrapure helium) 1.0 mL min^{-1}. The injector and interface temperature was 280 °C, with an ion source temperature of 200 °C. The oven temperature was programmed in the following sequence: initial temperature of 150 °C maintained for 2 min, increasing 10 ºC min^{-1} until the final temperature reaches 300 °C, continuing for 15 min. Mass spectra were acquired by electro-ionization at 70 eV by scanning in the range of *m/z* 40 to 550. The programming of the oven was based on the studies by Ferreira and Sanches Filho.[29]

The data were processed using the GC-MS solution 2.6 software (Shimadzu, Japan) and the compounds were identified using the NIST-05 library,[30] considering similarities greater than 80%.

Reagents and chemicals

For the development of the study, these sunscreen standards were used: octocrylene (OC), purity ≥ 98.00% HPLC and oxybenzone (BP-3), purity ≥ 98.00% GC, and hormone standards: estrone (E1), purity ≥ 99.00%, β-estradiol (E2), purity ≥ 98.00%, and estriol (E3), purity ≥ 97.00%.

The choice of analytes was based on the detection of this compound in other studies, with analysis of environmental samples and due to its high consumption by the population.[3,5,7-9,12,24,29,31]

A stock solution was prepared for each compound individually, using acetonitrile (ACN), purity grade 99.98% (Merck®, https://www.sigmaaldrich.com), for OC, BP-3 and E2. For E1 and E3, methanol was used, purity 99.80% (Synth® https://www.lojasynth.com). From the individual stock solutions, a Stock Standard Mix (SSM-5.0 mg L^{-1}) was prepared with the 5 analytes in ACN. Working solutions in dichloromethane

(DCM) were prepared from this solution, purity 99.99% (Synth® https://www.lojasynth.com). All solvents used were previously distilled.

Experimental

For validation of the chromatographic method, calibration curves were built in the range of 5.0 to 200.0 µg L^{-1} using at least 6 points and the 50 µg L^{-1} standard analyzed 4 times. Validation parameters were: linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and selectivity.[32,33]

Linearity

The method most used to correctly obtain this maximum proximity is the method of least squares, which provides unbiased results with minimal variance, within certain assumptions of a statistical nature. A linear relationship between a random variable *y* and a non-random variable *x* is described by Equation 1.[33]

$$y = \beta 0 + \beta 1x + \varepsilon \qquad \text{Equation 1}$$

where β0 and β1 are the model parameters, (linear coefficient-b, and angular coefficient-a) and *ε* is the random error associated with the determination of *y*

The significance analysis of the coefficients was obtained using the Statistica® software (STATSOFT, USA) using a significance level of 5% and a p-value lower than 0.05. A low p-value provides strong evidence that the obtained model is statistically significant.[34]

The validity of the results obtained is strongly dependent on the normality of the data analyzed. To verify the normality of responses, the Shapiro-Wilk normality test was applied.[33]

Finally, analysis of variance (ANOVA) was used to assess the significance of the fit of the model to the experimental data. For this, the value of the coefficient of determination (R^2) and Fisher's F tests were used. In addition, the relative error between values predicted by the model and experimental values was calculated, according to Equation 2.[33]

$$E\ (\%) = (Exp-P)/Exp \times 100 \qquad \text{Equation 2}$$

where E (%) is the error in %, Exp represent the value experience and P is the predicted value

Validation parameters were linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) and selectivity.[32]

Derivatization

The initial volume of Stock Standard Mix (SSM-5.0 mg L^{-1}) was derived, which when diluted to 1.0 mL corresponded to the concentrations of the standards used in the study of mass spectra, in the construction of calibration curves, and matrix effect. To the flasks, 0.5 mL of DCM, and 80.0 µL of *N*-trimethylsilyl-*N*-methyltrifluoroacetamide (MSTFA) were added at 80 ºC in a sand bath, for 30 minutes and 20.0 µL of pyridine (purity ≥ 99.50% - Merck® https://www.sigmaaldrich.com) as a catalyst for the silylation reaction[29] for 1 hour. Afterwards, the volume was completed with DCM at 1.0 mL and the sample was sent for analysis by GC-MS. The mass spectrometer operated in SCAN mode and with the Monitoring Ion Current resource (Monitoring Ion Current – MIC).

To evaluate the effect of derivatization on the chromatographic process, a standard of 1.0 mg L^{-1}, DCM, containing 3 hormones, E1, E2 and E3, and 2 sunscreens, BP-3 and OC, was chromatographed in SCAN mode with and without derivatization, defining the retention times and identification of the main ions in the mass spectrum. The derivatized compounds started to be described as: BP-3-TMS, E1-TMS, E2-TMS and E3-TMS.

The effect of silylation was evaluated by calculating the resolution between the analytes, and number of theoretical plates, according to Equations 3 and 4.[35]

$$R = 2\Delta Tr/(Wb_1 + Wb_2) \quad \text{Equation 3}$$

$$N = 16(Tr/Wb)^2 \quad \text{Equation 4}$$

where R is the resolution that describes the ability of a column to separate the peaks of interest and ΔTr is the difference in retention times (Tr_1-Tr_2); Wb is the base width of peaks 1 and 2 and N is the number of theoretical plates

From the study of the mass spectra (SCAN mode) the ions were selected for the quantification of each analyte in the SIM mode (Select Ion Monitoring).

Linearity was evaluated using the linear correlation coefficient (r) for each calibration curve. The LODs and LOQs were calculated based on the blank analysis (for each ion selected for quantification) considering 3 times the standard deviation of the blank signal for LOD and 10 times the standard deviation for LOQ, divided by the angular coefficient of the analytical curves referring to each analyte.[35]

Blanks were obtained from 0.5 mL of DCM derivatized with MSTFA, and pyridine as described in the derivatization process and the corrected volume to 1.0 mL with DCM.

Precision was determined from the repetition of derivatization and analysis of the 50.0 μg L^{-1} standard (n = 7), calculating the relative standard deviation expressed in %.[32,36]

Accuracy was evaluated using relative error, which is the difference between the value found from the analytical curves for the 100.0 μg L^{-1} standard.

To evaluate selectivity, an extract representing the matrix was added to 1000 μg L^{-1} of the mixture of standards, undergoing derivatization and analyzed in SCAN/SIM mode. The extract was obtained by LLE (Liquid Liquid Extraction) from domestic wastewater (DWW) collected at the entrance of the wastewater treatment plant (WTP) – Fragata, Pelotas (RS, Brazil).

To 100.0 mL of DWW, we added 10% NaCl and subjected to LLE with three portions of 15.0 mL of DCM.[31] The extracts were pooled and residual water was retained on anhydrous sodium sulfate columns. DCM extracts were concentrated with gentle N_2 flow, to less than 1.0 mL and derivatized with MSTFA. The volume was corrected to 1.0 mL and analyzed by GC-MS (SCAN/SIM mode). The compounds identification was performed by comparing the obtained spectra with those from the Library (NIST),[30] considering similarities greater than 80%.

Matrix effect was also evaluated through the recovery of the analytes added to the obtained LLE extract, comparing with a standard of 1000 μg L^{-1} as 100% and the standard deviation expressed as a percentage of the triplicates to evaluate the precision.

RESULTS AND DISCUSSIONS

Optimization of the chromatographic method

Effect of derivatization with MSTFA

Figure 1 shows the comparison between the chromatograms obtained in SCAN mode (Figure 1(a)) and with the aid of the monitored ion current tool (MIC-Monitoring Ion Current) (Figure 1(b)) for the standard mixture (SM) of the compounds at 1000 μg L^{-1} without derivatization. In Figure 1(a), the non-derivatized SM chromatogram showed only two peaks with a low response factor, suggesting coelutions. Through the mass spectrum, it was possible to identify the first peak as BP-3. The identification and detection of the other compounds was performed with the application of the current of monitored ions characteristic of each analyte (Figure 1(b)). Thus, an increase in the intensity of the signals can be observed and confirm the coelution of the analytes OC, E2 and E3.

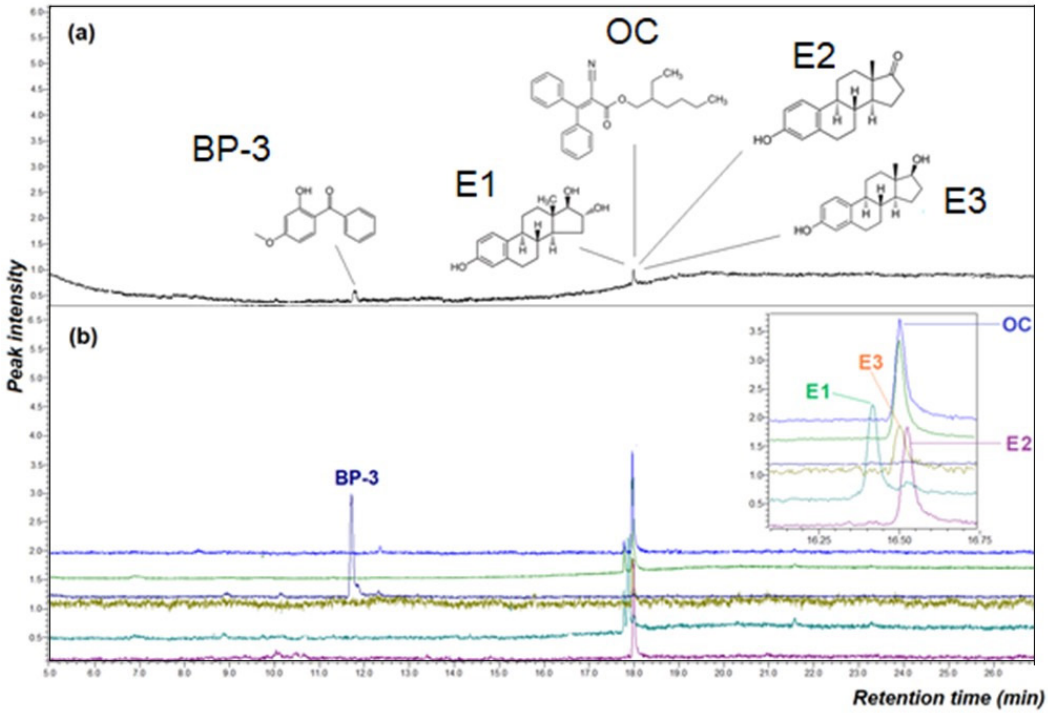

Figure 1. Chromatograms of the total ion (TIC) comparison obtained by the GC-MS analysis of not derivatizated sample. (a) SCAN mode and (b) SCAN mode with MIC.

After derivatization, as shown in Figure 2, it was possible to observe the presence of peaks referring to the 5 analytes present in the SM. The improvement in the chromatographic profile can be explained in terms of the structural modification caused by MSTFA. Silylation occurs through nucleophilic attack (SN_2) on the silylating agent.[37]

Consequently, molecules with such groups exchange a hydrogen for the trimethylsilyl group, generating TMS derivatives with higher molecular weight, lower polarity and, thus, favoring the interaction with the stationary phase used in this study. In general, there is an increase in the retention time and an increase in the response factor of the analytes.[37]

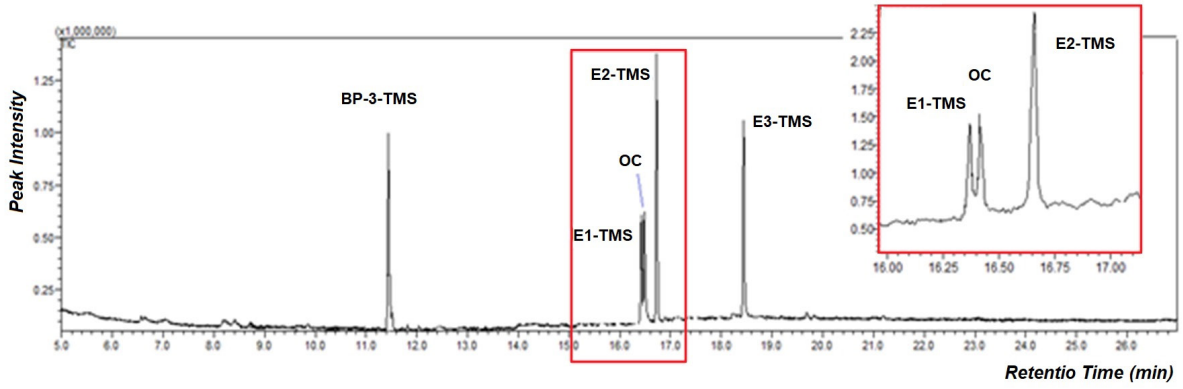

Figure 2. Chromatograms of the total ion (TIC) obtained by the GC-qMS analysis of derivatizated with MSTFA sample.

When comparing the number of theoretical plates (N) of the analytes before and after derivatization, an increase is generally observed, which means an improvement in the efficiency of the process, as shown in Figure 3.

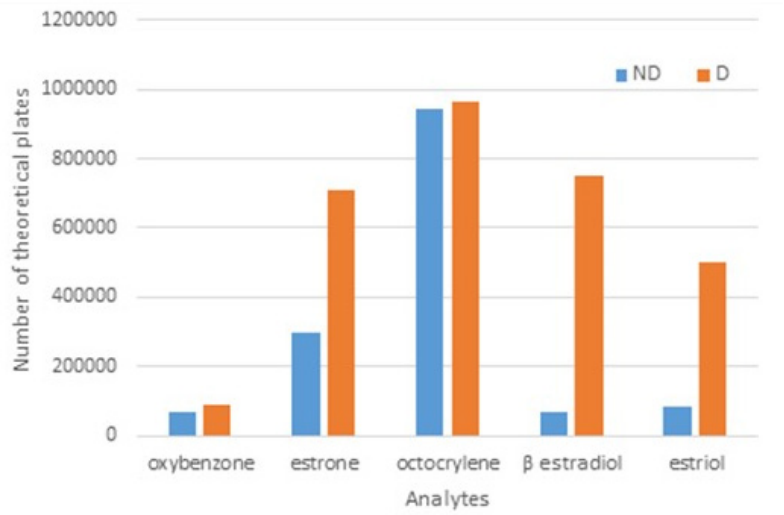

Figure 3. Number of theoretical plates of non-derivatized compounds *versus* derivatized compounds.

Such an increase is justified due to the decrease in the polarity of the compounds, which leads to a more effective interaction with the stationary phase, with an improvement in the resolution of the peaks as a whole, as shown in Figure 4.

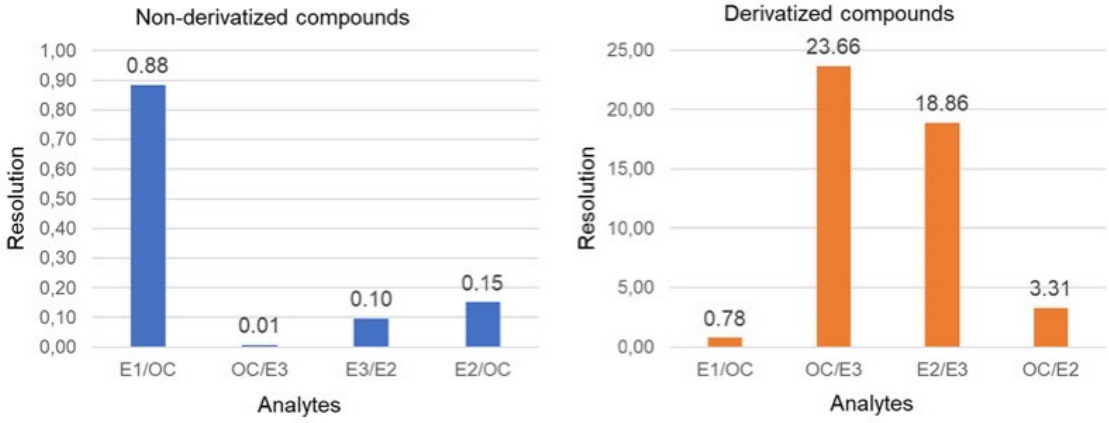

Figure 4. Resolution of the non-derivatized compounds *vs* derivatized compounds.

When evaluating the resolution between OC and E1-TMS, a reduction from 0.88 (without derivatization) to 0.78 (after derivatization) is observed. The reduction in this resolution occurs due to the increase in the molecular weight of E1-TMS and the consequent increase in its retention time, approaching the retention time of the OC which remains unchanged because it does not generate a TMS derivative. A value of 1 is the minimum for measurable separation and proper quantification, although 0.6 is sufficient to differentiate two peaks of the same height.[35]

The effect of derivatization on BP-3 increased the response factor, with a slight increase on N. For this analyte, the polarity remains active after the derivatization reaction, due to the presence of carboxyl and aldoxyl, which maintain the polar character, disfavoring the interaction with the low polarity stationary phase used in this work (polydimethylsiloxane with 5% phenyl groups). This behavior is evident when BP-3-TMS is compared with E3-TMS, which presents a blockade in the three polar hydroxyl groups (Figure 5),

increasing the interaction with the stationary phase and, subsequently, improving the resolution with OC, E1 and E2 and increasing N, as shown in Figures 3, 4 and 5.

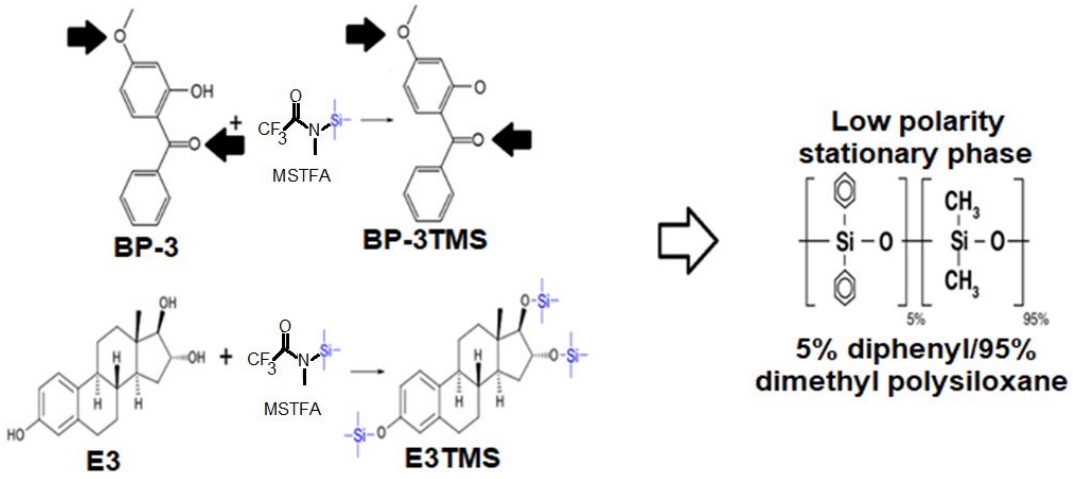

Figure 5. Oxybenzone and Estriol reaction with MSTFA in silylation process.

Seeking to increase sensitivity, reduce interference and achieve lower detection limits, an evaluation of the mass spectra in SCAN mode (Figure 6) was carried out to select the ions to be used for analyzes in SIM mode.

Loss of trimethylsilanol (TMSOH) is a typical feature observed in many GC-MS spectra of different hydroxy steroids. This loss depends on the steric characteristics of the steroid skeleton, as well as on the availability of hydrogen.[38]

Observing the E1-TMS mass spectrum, we verified as more abundant mass fragments, the molecular ion 342 $[M]^+$ (base peak), 257 $[M - 85]^+$, 327 $[M - 15]^+$ due to the loss of a methyl group, and an ion with *m/z* 218.[39] The E1-TMS also has the possibility of an intramolecular transfer of hydrogen to the ketone group ring (C17) with the formation of *m/z* 285 referring to the $[M\text{-}57]^+$.[21,40] On the other hand, it presents a mechanism for the formation of the *m/z* 286 ion ($[M - 56]^+$) of lesser intensity due to the simultaneous loss of CO and C_2H_2. The low-intensity *m/z* 163 ion appears both in E1-TMS and E2-TMS, and to a lesser intensity in E3-TMS and can be attributed to the fragment $[(CH_3)_2Si–O–C_6H_3–CH_2]^+$ relative to the ring with a phenolic group.[39] Both E2-TMS and E3-TMS presented the ion *m/z* 73 as more intense referring to the $[TMS]^+$, since they present this group in greater proportion according to the structures shown in Figure 6.

In the E2-TMS mass spectrum, 416 $[M]^+$ (molecular peak) and 285 $[M\text{-}(C_3H_5\text{+TMSOH})]^+$ ions stand out, in addition to ions 326, $[M\text{-TMSOH}]^+$ and 401, $[M\text{-}CH_3]^+$. In the E3-TMS spectrum we can see fragments 504, $[M]^+$ (molecular peak), 386, $[M\text{-}(C_2H_4\text{+TMSOH})]^+$ and, the íon 147 $[(CH_3)_3Si\text{-}O\text{-}Si(CH_3)_2]^+$ formed by the TMS groups in neighboring positions.[38,40]

The cleavage of the methyl radical of a TMS group followed by a neutral loss of the remaining dimethylsilyloxy group can explain the 311 $[M – CH_3 – 2x(CH_3)_2SiO$ fragments, present in E3-TMS. The *m/z* 129 $[CH_3SiOC_3H_6]^+$ is found in both E2-TMS and E3TMS and is considered an indicator of additional TMS groups TMS.[38,40]

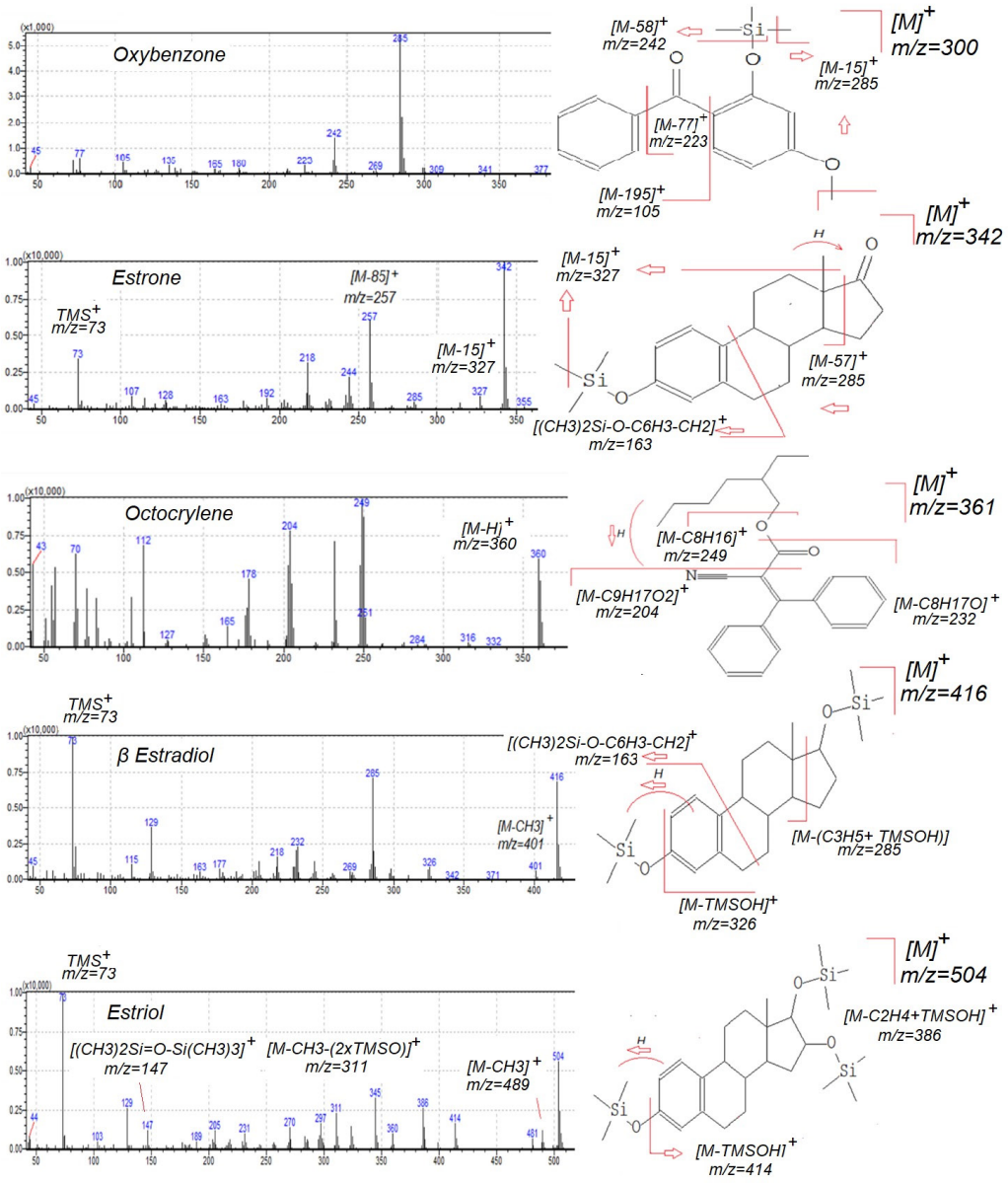

Figure 6. Mass spectra in SCAN mode for hormones and UV filters (TMS).

In the mass spectra of the sunscreens, we observed for BP-3-TMS, the 300 $[M]^+$ ions (molecular peak) of lower intensity, the base ion 285 $[M-15]^+$, and the ions 242 $[M-58]^+$, 223 $[M-77]^+$ and 105 $[M-195]^+$, generated by α carbonyl cleavages (Figure 6). The OC presents the molecular ion of 361, odd characteristic of nitrogenous compounds, which converts to 360 by loss of a hydrogen. The possibility of a McLafferty rearrangement causes a hydrogen from the alkyl group to migrate to the carbonyl group, releasing the neutral molecule C_8H_{16} and generating the base *m/z* 249.[32] Alternatively, bond breaks between carbonyl and oxygen lead to the formation of ion 232, and between carbonyl and carbon to ion 204.[37,39]

Considering the specificity, relative intensities, of the mass fragments of each analyte, the ions were selected for quantification and confirmation in SIM mode. Combining the retention times of the TMS derivatives and the selected ions, time windows were determined to monitor different ions (Table I), avoiding interference, and improving selectivity.

Table I. Chromatographic conditions for quantitative analysis in SIM mode

Compound	Quantification ion	Tr (min)	Confirmation Ion	Time window (min)
BP-3-TMS	285	11.505	285; 300	5 -16
E1-TMS	342	16.426	257; 342	16 - 27
OC	249	16.528	249; 361	16 - 27
E2-TMS	416	16.801	285; 416	16 - 27
E3-TMS	504	18.601	311; 386; 504	16 - 27

Method validation

Table II presents the merit parameters for the chromatographic method in SIM mode.

Table II. Parameters for analytical methodology validation

Compound	RT (min)	a	b	r	Accuracy (ER%)	Precision (RSD%)	LOD (μg L^{-1})	LOQ (μg L^{-1})
BP-3-TMS	11.505	15.1	289.1	0.999	8.6	3.2	1.3	4.4
E1-TMS	16.426	5.8	-81.5	0.998	4.5	4.8	2.1	7.0
OC	16.528	4.5	-110.7	0.998	5.0	4.2	2.9	9.6
E2-TMS	18.801	8.9	56.2	0.990	4.9	5.5	2.5	8.4
E3-TMS	18.601	7.3	185.8	0.995	10.0	10.8	2.5	8.5

a: Angular coefficient; b: linear coefficient; RSD: relative standard deviation expressed as a percentage; ER%: relative error expressed as a percentage; LOD: limit of detection; LOQ: limit of quantification.

After analyzing the results for the angular coefficient (a), a greater sensitivity of the analytical method for oxybenzone was observed, followed by β-estradiol and estriol. The equipment presented a linear response, with correlation coefficients with results greater than 0.9, required by INMETRO[32] for validating a method.

Accuracy and precision were acceptable for all analytes, with ER% between 4.5 and 10.0 % and RSD% between 3.2 and 10.8%. Since the analytes were evaluated in the range of μg L^{-1}, which admits RSD% up to 20% and ER% up to 15%.[29] The results showed agreement with those parameters required by the United States Environmental Protection Agency.[23]

The LODs and LOQs values ranged from 1.3 to 2.9 μg L^{-1} and 4.4 to 9.6 μg L^{-1}, respectively. These values were in accordance with those described by Ferreira and Sanches Filho.[29]

Montagner[25] describes hormone levels in Brazil ranging between 0.56 and 9,717 ng L^{-1} for raw sewage and 0.09 and 2,080 ng L^{-1} for treated sewage. Levels of 17β-estradiol have been described in WWTPs and rivers in North America in the ranges of 1 – 22 ng L^{-1} and 0 – 4.5 ng L^{-1}, respectively,[41] while sunscreens, such as Benzophenones have been determined in the ranges of 10 ng L^{-1} to 80 ng L^{-1} in wastewater.[5] Considering the values of LODs, LOQ and ECs concentrations described in real samples, pre-concentration methods should be used for determinations of these analytes in samples of this type.

Chromatographic characterization of LLE extract of DWW

Table III presents chromatographic characterization of the extract obtained by LLE from raw domestic wastewater followed by derivatization and analysis by GC-MS (SCAN). The area percentage composition of the chemical classes and number of Compounds are shown in Figure 7.

It is observed that the matrix is a complex mixture, with the majority presence of organic acids (57.7%), followed by hydrocarbons (16.2%) and fatty alcohols (8.85%). Among the ECs found in the DWW sample, drugs (caffeine, paracetamol, ibuprofen), plasticizer residue (Phthalates) and steroidal hormones (androsterone, androstanedione and progesterone) stood out. Cholesterol and coprosterol were found in the class of steroid nucleus - cyclopentane perhydro phenathene (CPPP).

The study of the matrix obtained from DWW and fortified with ECs allowed the detection of some coelutions, forcing the reprogramming of the oven temperature of the gas chromatograph, as well as the inclusion of new ions in SIM mode.

Table III. Chemical identification of the DWW by GC-MS

Compound	Retention time (min)	Relative área %	Chemical classes
Decanoic acid	10.249	1.00	Carboxylic Acid
Methyl 10-oxoundecanoate	11.195	0.19	Ester
1,3,5-Cycloheptatriene, 7,7-dimethyl-3,4-dihidróxi-	11.642	0.85	Alcohol
Dodecanol	12.016	0.60	Alcohol
methyl 2-(4-isobutylphenyl)propanoate	12.966	0.38	Ester
N-(4-hydroxyphenyl)acetamide	13.147	0.12	Nitrogenous compounds
Dodecanoic acid	13.293	2.77	Carboxylic Acid
Di-n-decylsulfone	14.783	0.23	Sulfur compounds
Tetradecanol	14.891	0.43	Alcohol
Tetradecanoic acid	16.074	3.00	Carboxylic Acid
Caffeine	16.33	0.95	Nitrogenous compounds
1,2-Benzenedicarboxylic acid, *bis*(2-methylpropyl) ester	16.467	0.58	Phthalate (Ester)
Pentadecanoic acid	17.372	1.02	Carboxylic Acid
Hexadecanol	17.523	1.90	Alcohol
Sulfurous acid, octadecyl 2-propyl ester	17.654	0.19	Sulfur compounds
cis-9-Hexadecenoic acid	18.318	0.98	Carboxylic Acid
trans-9-Hexadecenoic acid	18.382	3.82	Carboxylic Acid
Hexadecanoic acid	18.616	14.99	Carboxylic Acid
Methyl 11-octadecenoate	19.322	0.46	Ester
Octadec-9Z-enol	19.647	0.80	Alcohol
1-Octadecanol	19.925	1.77	Alcohol
9,12-Octadecadienoic acid	20.624	3.81	Carboxylic Acid
cis-9-Octadecenoic acid	20.671	5.33	Carboxylic Acid
trans-9-Octadecenoic acid	20.743	2.13	Carboxylic Acid

(continues on the next page)

Table III. Chemical identification of the DWW by GC-MS (continuation)

Compound	Retention time (min)	Relative área %	Chemical classes
Octadecanoic acid	20.944	8.70	Carboxylic Acid
9,12-Octadecadienoic acid (Z,Z)-	21.154	0.78	Carboxylic Acid
18-Methyl-nonadecanol	22.134	0.80	Alcohol
6.*α*.-Hydroxy-progesterone	24.078	0.10	CPPP
1-Docosanol	24.175	0.81	Alcohol
Di-n-octyl phthalate	24.316	0.48	Phthalate (Ester)
trans-Androsterone	25.169	0.08	CPPP
Androstane-11,17-dione, 3-[(hidroxy]-, (3.*α*.,5.*β*.)-	25.252	0.21	CPPP
Octacrileno	25.679	0.01	Nitrogenous compounds
Coprostan-3-ol	29.658	0.26	CPPP
Cholesterol	30.875	0.87	CPPP
16-Hentriacontanone	32.213	1.06	Ketone
Lithocholic acid	32.326	0.86	CPPP

CPPP: compounds with a nucleus cyclopentane perhydro phenanthrene

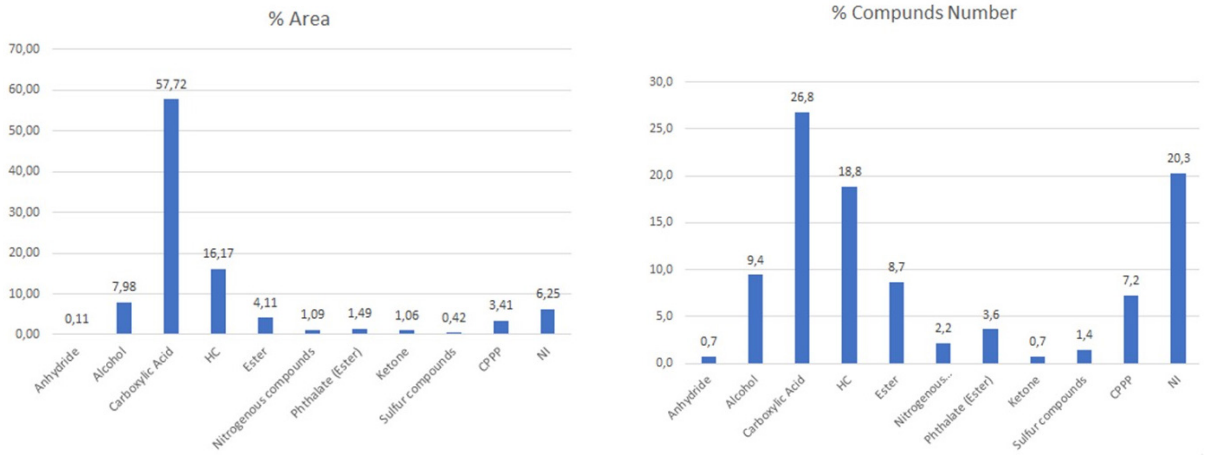

Figure 7. Percentage composition of area and number of compounds, according to chemical classes in DWW.

Figure 8 (a and b) shows the comparison of the chromatograms in SCAN mode for the DWW extracts, fortified and non-fortified. This image highlights the coelution that occurred for BP-3-TMS with a compound that has the 285 ion in common. In Figure 8 (c; d; e) we can observe the mass spectra for fortified and unfortified samples, and BP-3-TMS standard, illustrating the interference, by coelution, in the mass spectra. This situation would lead to errors, both in the confirmation of the compound in SCAN mode and in the quantification in SIM mode. In this way, the *m/z* 242 ion was selected, specific to BP-3-TMS as we can see in the presented mass spectra. The spectrum in Figure 9 (d) does not show *m/z* 242.

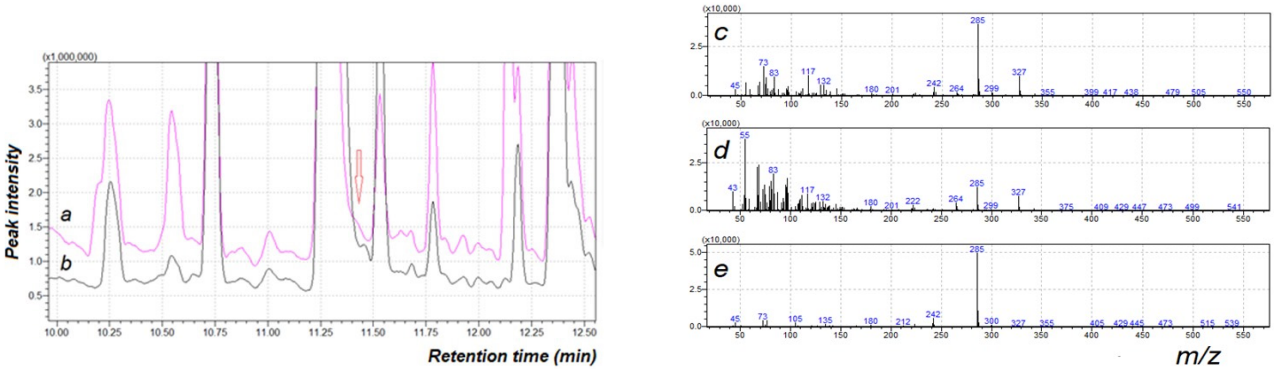

Figure 8. Comparison between chromatograms (a) DWW matrix fortified (b) DWW matrix non-fortified; (c) Mass spectra fortified sample; (d) Unfortified sample spectrum (e) Standard Spectrum BP-3-TMS.

A similar situation was observed for estriol in relation to *m/z* 504, which is also present in fragments of the stationary phase, which may interfere with the method adopted for the quantification of this Compound (Table III). Thus, the *m/z* 386 ion was selected for quantification.

Table IV presents the chromatographic conditions redefined for the quantitative method in SIM mode.

Table IV. Chromatographic conditions optimized for quantitative analysis in SIM mode

Temperature program	**Initial temperature of 100 °C held 2 min then heating at 8 °C min^{-1} to 300 °C held for 13 min**			
Compound	**Quantification ion**	**Tr (min)**	**Confirmation ion**	**Time window (min)**
BP-3-TMS	242.00	19.416	242; 285; 300	5 -22
E1-TMS	342.00	25.578	257; 342	22-39
OC	249.00	25.675	249; 361	22-39
E2-TMS	416.00	25.962	285; 416	22-39
E3-TMS	386.00	27.976	311; 386; 504	22-39

In Figure 9, we can see the improvement in selectivity combining oven temperature programming and mass spectrometer analysis in SIM mode for the newly selected ions (242 for oxybenzone and 386 for estriol) for quantification in SIM mode. The new programming of the oven led to an improvement in the resolution of the BP-3-TMS, allowing the quantification of both 242 and 285 ions.

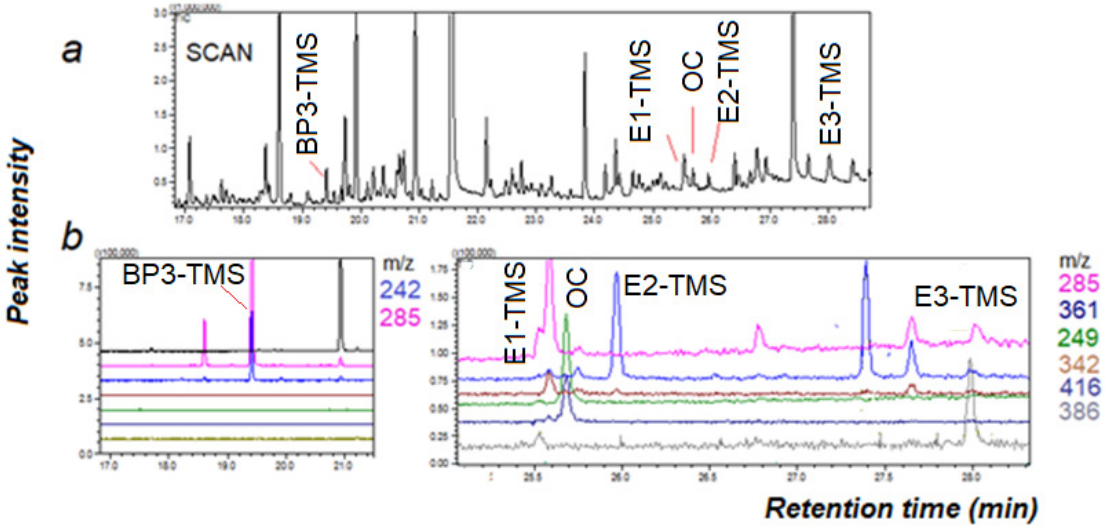

Figure 9. Chromatogram for derivatized sample analyzed by GC-MS in SCAN mode (a) and SIM mode (b) according to the new chromatographic conditions in Table IV.

Linearity, precision, accuracy, matrix effect, limit of detection and limit of quantification studies were developed for validation based on the new chromatographic conditions. The results including statistical treatments can be seen in Table V.

The data presented a normal distribution with W values (Shapiro-Wilk test) of 0.785 to 0.888. These values are higher than the critical W value set at a significance level of 10% and a number of observations of 9, which is 0.764.

The low p values confirm that the regression coefficients are significant. We can see in Table V that the F calculated for the ratio between the mean squares (MS) of the regression and the pure error MS was higher than the F tabulated, with degrees of freedom (1:3), for all analytes. This confirms the linear relationship between the two variables, as well as the F calculated from the ratio of the MS of the lack of fit and the MS of the pure error was smaller than the F tabled, for the degrees of freedom (4:3), demonstrating a model without lack of fit. The determination and adjustment coefficients can explain more than 99.9% of the result overall. Considering this data. we can say that the models are linear, significant and predictive. for all analytes.

Both the RSD values calculated for the models (<1.8%) and those calculated for the repeatability of the derivatization process (<7.2%) met the analytical quality requirements for the working range (ug L^{-1}) according to the INMETRO (20%).[32]

The accuracy of the models was confirmed by the high similarity between experimental and predicted values, and low relative errors, for each concentration level, while the accuracy of the derivatization process as a whole, was confirmed by the low relative errors obtained by the triplicate analysis of the standard 100 ug L^{-1}, less than 2%.

The LOD and LOQ are in agreement in Ferreira & Sanches Filho[29] and higher than those described by He *et al.*[14] The precision is equivalent to the RSD values described by He *et al.,*[14] for the same analytes and to the values described for oxybenzone and octraclene by Ashfaq *et al.*[42] The work carried out by the cited authors used LC-MS/MS equipment less available in environmental analysis laboratories when compared to GC-MS.

The selectivity and achievement of the matrix was confirmed by the quality of the mass spectra obtained in SCAN mode, that presented similarities greater than 80% and also by the recoveries in the range of 95.90% for E3-TMS to 114.60% for E1-TMS and the precisions expressed by the RSD were less than 6.0%. These results are in accordance with INMETRO requirements for method validation too.[32]

Table V. Parameters for validation of analytical methodology

	BP-3-TMS/285.00		BP-3-TMS/242.00		E1-TMS/342.00		OC/249.00		E2-TMS/416.00		E3-TMS/386	
Coefficient	**Value**	**p**	**Value**	**p**	**Value**	**p**	**Value**	**p**	**Value**	**p**	**Value**	**p**
b	1195.661	0.000204	187.18519	1.761E-06	282.9524	2.121E-05	287.7037	4.071E-06	299.7090	8.119E-06	396.6878	1.093E-07
a	99.339	6.46E-07	15.076543	6.078E-09	7.6397	1.946E-06	7.4272	4.269E-07	22.1400	3.635E-08	9.0256	1.673E-08
R^2	0.99905		0.99993		0.998		0.99877		0.99996		0.9992	
r	0.999		0.999		0.999		0.999		0.999		0.999	
adj	0.99891		0.99992		0.99977		0.9986		0.99995		0.9991	
$F_{calculated}$ model	22669.66	1.946E-06	508738.11	6.078E-09	10867.92	1.946E-06	29882.73	4.269E-07	154407.1	3.635E-08	259000.2	1.673E-08
$F_{calculated}$ lack of fit	4.64	0.116973	7.6076998	0.0635865	4.70	0.1169729	8.44	0.055460	0.9	0.556190	4.4	0.125518
RSD%*	1.8		0.4		1.8		1.1		0.7		0.4	
RSD%**	4.9		2.4		7.2		4.7		3.1		5.1	
$W_{calculated}$	0.785		0.785		0.888		0.795		0.787		0.870	
Concentration levels	**RE %***		**RE %***		**RE %***		**RE %***		**RE %***		**RE %***	
5	7.3		-3.4		-0.4		5.5		2.1		2.0	
10	-13.1		2.3		-0.4		5.8		1.4		0.4	
25	-2.7		-1.1		-8.2		-5.3		-0.8		0.1	
50	0.9		0.4		1.1		-1		-0.1		-0.3	
100	-1.2		-0.7		2.8		1.4		-0.4		-0.4	
200	-0.3		0.1		-0.9		0.1		0.1		0.2	
	ER%**		ER%**		ER%**		ER%**		ER%**		ER%**	
ER%**100	1.7		1.0		3.8		1.2		-1.9		-2.4	
Matrix effect % Recovery ± RSD%	111.2±5.8		110.5±5.4		114.6±5.3		104.9±4.1		107.3±3.7		95.9±0.6	
LOD/LOQ (µg L^{-1})	0.1	0.3	0.8	2.6	1.3	4.2	1.3	4.2	0.4	1.4	0.9	2.9

a: Angular coefficient; b: linear coefficient; RSD%*: Relative standard deviation to repeat readings of the 50 ug L^{-1} s; RSD%**: Relative standard deviation to the repetition of the derivatization procedure and analyzes of the 50 ug L^{-1} point (n=7); ER%*: relative error as a function of the observed and predictive value by the statistical model ; ER%**: relative error expressed as a percentage of the derivatization and analysis procedure (n=3) of the 100 ug L^{-1}; LOD: limit of detection; LOQ: limit of quantification. $F_{reference}$ for the regression (1.3) = 10.13; $F_{reference}$ (4.3) = 9.12. Table F-Fischer in the level of 95%; the critical W value set at a significance level of 10 % (n= 9) = 0.764; R2 – coefficient of determination.

CONCLUSIONS

It is concluded that the derivatization reaction with MSTFA in the samples analyzed by GC-MS led to a reduction in the polarity of the compounds. resulting in greater interactions with the stationary phase, promoting an improvement in the resolution. in the response factor for the TMS derivatives. The method developed for the qualitative analysis (SCAN mode) allowed the identification of the analytes more accurately. with similarities of the spectra superior to 80%. The chromatographic method optimized in SIM mode for the quantification of the analytes proved to be precise, exact, with a linear response, and with selectivity in accordance with the requirements and standards of the regulatory bodies. However, the LODs and LOQs suggest the need for a pre-test concentration method for the quantitative determination of these analytes in real samples. such as domestic wastewater. The chromatographic method proved to be suitable for initial screening of ECs in extracts obtained from samples of domestic sewage. being a tool for monitoring the levels of these contaminants in wastewater treatment plant (WTP).

Conflicts of interest

The authors declare that there is no conflict of interest.

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REFERENCES

(1) Varsha, M.; Kumar, P. S.; Rathi, B. S. A review on recent trends in the removal of emerging contaminants from aquatic environment using low-cost adsorbents. *Chemosphere* **2022**, *287*, 132270. https://doi.org/10.1016/j.chemosphere.2021.132270

(2) Cartaxo, A. S. B.; Albuquerque, M. V. C.; Silva, M. C. C. P.; Rodrigues, R. M. M.; Ramos, R. O.; Sátiro, J. R.; Lopes, W. S. Contaminantes emergentes presentes em águas destinadas ao consumo humano: Ocorrência. Implicações e Tecnologias de Tratamento. *Brazilian Journal of Development* **2020**, *6*, 61814-61827. https://doi.org/10.34117/bjdv6n8-559

(3) Guesmi, A.; Ohlund, L.; Sleno, L. In vitro metabolism of sunscreen compounds by liquid chromatography/high-resolution tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2020**, *34*, e8679. https://doi.org/10.1002/rcm.8679

(4) Negreira, N.; Rodríguez, I.; Ramil, M.; Rubí, E.; Cela, R. Solid-phase extraction followed by liquid chromatography–tandem mass spectrometry for the determination of hydroxylated benzophenone UV absorbers in environmental water samples. *Anal. Chim. Acta* **2009**, *654*, 162-170. https://doi.org/10.1016/j.aca.2009.09.033

(5) Pamplona-Silva, M. T.; Mazzeo, D. E. C.; Bianchi, J.; Marin-Morales, M. A. Estrogenic compounds: chemical characteristics, detection methods, biological and environmental effects. *Water Air Soil Pollut.* **2018**, *229*, 144. https://doi.org/10.1007/s11270-018-3796-z

(6) Adeel, M.; Song, X.; Wang, Y.; Francis, D.; Yang, Y. Environmental impact of estrogens on human, animal and plant life: A critical review. *Environ. Int.* **2017**, *99*, 107-119. https://doi.org/10.1016/j.envint.2016.12.010

(7) Cais, T. A.; Procópio, A. M. S.; Kondo, M. M.; Silva, F. S.; de Andrade, S. J. Analytical procedures for the determination of estrogen compounds in a surface water reservoir in Southeast Brazil. *Ecletica Quim. J.* **2021**, *46* (1), 41-51. https://doi.org/10.26850/1678-4618eqj.v46.1.2021.p41-51

(8) He, K.; Hain, E.; Tarnowskid, T.; Blaney, L. Occurrence of antibiotics, estrogenic hormones, and UV-filters in water, sediment, and oyster tissue from the Chesapeake Bay. *Sci. Total Environ.* **2019**, *650*, 3101-3109. https://doi.org/10.1016/j.scitotenv.2018.10.021

(9) Schneider, S. L.; Lim, H. W. Review of environmental effects of oxybenzone and other sunscreen active ingredients. *J. Am. Acad. Dermatol*. **2019**, *80* (1), 266-271. https://doi.org/10.1016/j.jaad.2018.06.033
(10) Raffa, R. B.; Pergolizzi, J. V.; Taylor, R.; Kitzen, J. M. Sunscreen bans: Coral reefs and skin cancer. *J. Clin. Pharm. Ther.* **2019**, *44* (1), 134-139. https://doi.org/10.1111/jcpt.12778
(11) Ramos, S.; Homem, V.; Alves, A.; Santos, L. A review of organic UV-filters in wastewater treatment plants. *Environ. Int.* **2016**, *86*, 24-44. https://doi.org/10.1016/j.envint.2015.10.004
(12) Teixeira, R. B.; Marques, C. A.; Carvalho, N. R.; Gomes, L. E. T.; Silva, F. T.; Paiva, T. C. B. Determination of estrogenic hormones in sewage and effluent of a decentralized sewage treatment plant by activated sludge. *Rev. Ambient. Água* **2018**, *13* (2). https://doi.org/10.4136/ambi-agua.2059
(13) Ashfaq, M.; Li, Y.; Zubair, M.; Ur Rehman, M. S.; Sumrra, S. H.; Nazar, M. F.; Mustafa, G.; Fazal, M. T.; Ashraf, H.; Sun, Q. Occurrence and risk evaluation of endocrine-disrupting chemicals in wastewater and surface water of Lahore, Pakistan. *Environ. Geochem. Health* **2023**, *45* (7), 4837-4851. https://doi.org.10.1007/s10653-023-01527-6
(14) He, K.; Timm, A.; Blaney, L. Simultaneous determination of UV-filters and estrogens in aquatic invertebrates by modified quick, easy, cheap, effective, rugged, and safe extraction and liquid chromatography tandem mass spectrometry. *J. Chromatogr. A.* **2017**, *1509*, 91-101. https://doi.org/10.1016/j.chroma.2017.06.039
(15) Sun, Q.; Li, Y.; Li, M.; Ashfaq, M.; Lv, M.; Wang, H.; Hu, A.; Yu, C. P. PPCPs in Jiulong River estuary (China): spatiotemporal distributions, fate, and their use as chemical markers of wastewater. *Chemosphere*, **2016**, *150*, 596-604. https://doi.org/10.1016/j.chemosphere.2016.02.036
(16) Hajeb, P.; Zhu, L.; Bossi, R.; Vorkamp, K. Sample preparation techniques for suspect and non-target screening of emerging contaminants. *Chemosphere* **2022**, *287*, 132306. https://doi.org/10.1016/j.chemosphere.2021.132306
(17) Queiroz, M. E. C.; Acquaro Junior, V. R. Cromatografia Líquida no modo Column Switching para Bioanálises. *Scientia Chromatographica* **2017**, *9* (2), 73-82. http://dx.doi.org/10.4322/sc.2017.007
(18) Arsand, J. B. *Avaliação ambiental da presença de contaminantes emergentes em água de rio utilizando espectrometria de massa*. Ph.D. Dissertation. Universidade Federal do Rio Grande do Sul. Porto Alegre, RS, Brazil, 2019. http://hdl.handle.net/10183/197230
(19) Goeury, K.; Duy, S. V.; Munoz, G.; Prévost, M.; Sauvé, S. Data supporting the optimization of liquid chromatography tandem mass spectrometry conditions to analyze EPA priority hormones and bisphenol A in water samples. *Data Brief* **2019**, *24*, 103958. http://doi.org/10.1016/j.dib.2019.103958
(20) Chaves, M. D. J. S.; Barbosa, S. C.; Malinowski, M. M.; Volpato, D.; Castro, Í. B.; Franco, T. C. R. S.; Primel, E. G. Pharmaceuticals and personal care products in a Brazilian wetland of international importance: Occurrence and environmental risk assessment. *Sci. Total Environ.* **2020**, *734*, 139374. https://doi.org/10.1016/j.scitotenv.2020.139374
(21) Medeiros, P. M.; Simoneit, B. R. Gas chromatography coupled to mass spectrometry for analyses of organic compounds and biomarkers as tracers for geological. environmental. and forensic research. *J. Sep. Sci.* **2007**, *30*, 1516-1536. https://doi.org/10.1002/jssc.200600399
(22) Zhou, Y.; Zi-Jian, W.; Ning, J. I. A. Formation of multiple trimethylsilyl derivatives in the derivatization of 17α-ethinylestradiol with BSTFA or MSTFA followed by gas chromatography-mass spectrometry determination. *J Environ Sci.* **2007**, 19, 879-884. https://doi.org/10.1016/s1001-0742(07)60146-6
(23) United States Environmental Protection Agency (EPA). Method 1698: *Steroids and Hormones in Water. Soil. Sediment. and Biosolids by HRGC/HRMS*. 2007. https://www.epa.gov/sites/default/files/201510/documents/method_1698_2007.pdf (accessed 2022-06-15).
(24) Choi, M. H.; Kim, K. R.; Chung, B. C. Determination of estrone and 17 β-estradiol in human hair by gas chromatography-mass spectrometry. *Analyst* **2000**, *125*, 711-714. https://doi.org/10.1039/a909107f

(25) Montagner, C. C.; Vidal, C.; Acayaba, R. D. Contaminantes emergentes em matrizes aquáticas do Brasil: cenário atual e aspectos analíticos. ecotoxicológicos e regulatórios. *Quím. Nova* **2017**, *40* (9), 1094-1110. https://doi.org/10.21577/0100-4042.20170091

(26) Sghaier, R. B.; Net, S.; Ghorbel-Abid, I.; Bessadok, S.; Le Coz, M.; Hassan-Chehimi, D. B.; Trabelsi-Ayadi, M.; Tackx, M.; Ouddane, B. Simultaneous detection of 13 endocrine disrupting chemicals in water by a combination of SPE-BSTFA derivatization and GC-MS in transboundary rivers (France-Belgium). *Water Air Soil Pollut.* **2017,** *228*, 2. https://doi.org/10.1007/s11270-016-3195-2

(27) Thurman, E. M.; Writer, J. H.; Ferrer, I. Injection port derivatization for GC/MS–MS: analysis of hormones in water. *Compr. Anal. Chem.* **2013**, *61*, 115-141. https://doi.org/10.1016/B978-0-444-62623-3.00005-8

(28) Moon, J. Y.; Kim, K. J.; Moon, M. H.; Chung, B. C.; Choi, M. H. A novel GC-MS method in urinary estrogen analysis from postmenopausal women with osteoporosis [S]. *J. Lipid Res*. **2011**, *52* (8), 1595-1603. https://doi.org/10.1194/jlr.D016113

(29) Ferreira, F. S.; Sanches Filho, P. J. Otimização e validação de metodologia para determinação de hormônios sexuais em matrizes aquosas por SPE e GC/MS: determinação de hormônios sexuais em matrizes aquosas. *Revista Ibero-Americana de Ciências Ambientais* **2020**, *11* (5), 500-509. https://doi.org/10.6008/CBPC2179-6858.2020.005.0045

(30) National Institute of Standards and Technology (NIST). Mass Spectrometry Data Center. http://chemdata.nist.gov

(31) Wojnarowski, K.; Podobiński, P.; Cholewińska, P.; Smoliński, J.; Dorobisz, K. Impact of Estrogens Present in Environment on Health and Welfare of Animals. *Animals* **2021**, *11* (7), 2152. https://doi.org/10.3390/ani11072152

(32) Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO). *Orientação sobre validação de métodos analíticos*. DOQ-CGCRE-008, 2016. http://www.inmetro.gov.br/Sidoq/Arquivos/Cgcre/DOQ/DOQ-Cgcre-8_05.pdf (Acessed 2022-06-15).

(33) Neto, B. B.; Scarminio, I. S.; Bruns, R. E. *Como fazer experimentos: pesquisa e desenvolvimento na ciência e na indústria*. 4th ed. Bookman, Porto Alegre, RS, 2010. Chapter 2, pp 25-69. ISBN 978-85-7780-713-0.

(34) Gupta, S.; Gupta, G. K.; Mondal, M. K. Slow pyrolysis of chemically treated walnut shell for valuable products: Effect of process parameters and in-depth product analysis. *Energy J.* **2019**, *181*, 665-676. https://doi.org/10.1016/j.energy.2019.05.214

(35) Lanças, F. M. *Validação de métodos cromatográficos de análise*. 6th ed. RiMa, São Carlos, SP, 2004. Chapter 2, pp 16-20.

(36) Arduim, J.; Hackbart, H. C. S.; Amaral, M. A. F. S.; Sanches Filho, P. J. Otimização de metodologia para análise de hidrocarbonetos em sedimentos lagunares. *Rev. Ibero-Am. Ciênc. Ambient.* **2019**, *10* (3), 165-178. http://doi.org/10.6008/CBPC2179-6858.2019.003.0015

(37) Schummer, C.; Delhomme, O.; Appenzeller, B. M.; Wennig, R.; Millet, M. Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. *Talanta* **2009**, *77* (4), 1473-1482. https://doi.org/10.1016/j.talanta.2008.09.043

(38) Kollmeier, A. S.; de la Torre, X.; Müller, C. Botrè, F.; Parr, M. K. In-depth gas chromatography/tandem mass spectrometry fragmentation analysis of formestane and evaluation of mass spectral discrimination of isomeric 3-keto-4-ene hydroxy steroids. *Rapid Commun. Mass Spectrom.* **2020**, *4*, e8937. https://doi.org/10.1002/rcm.8937

(39) Shareef, A.; Parnis, C. J.; Angove, M. J.; Wells, J. D.; Johnson, B. B. Suitability of N. O-bis (trimethylsilyl) trifluoroacetamide and N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide as derivatization reagents for the determination of the estrogens estrone and 17α-ethinylestradiol by gas chromatography–mass spectrometry. *J. Chromatogr. A*. **2004**, *1026* (1-2), 295-300. https://doi.org/10.1016/j.chroma.2003.10.110

(40) Harvey, D. J.; Vouros, P. Mass spectrometric fragmentation of trimethylsilyl and related alkylsilyl derivatives. *Mass Spectrom. Rev.* **2020**, *39* (1-2), 105-211. https://doi.org/10.1002/mas.21590
(41) Pal, A.; Gin, K. Y. H.; Lin, A. Y. C.; Reinhard, M. Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences. sources. fate and effects. *Sci. Total Environ.* **2010**, *408* (24), 6062-6069. https://doi.org/10.1016/j.scitotenv.2010.09.026
(42) Ashfaq, M.; Li, Y.; Rehman, M. S. U.; Zubair, M.; Mustafa, G.; Nazar, M. F.; Yu, C. P.; Sun, Q. Occurrence, spatial variation and risk assessment of pharmaceuticals and personal care products in urban wastewater, canal surface water, and their sediments: A case study of Lahore, Pakistan. *Sci Total Environ*. **2019**, *688*, 653-663. https://doi.org/10.1016/j.scitotenv.2019.06.285